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Fabricating Microarrays of Functional Proteins Using Affinity Contact Printing

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Supporting Information

Proteins were from Sigma and used as received. Antibodies were labelled either with tetramethylrhodamine isothiocyanate (TRITC) or fluorescein isothiocyanate (FITC). Solutions of proteins and adsorption experiments were done in PBS or in PBS-containing 1% BSA. Bis(sulfosuccinimidyl)suberate (BS^3) was from Pierce, aminopropyltriethoxysilane (APTS) from Fluka. PDMS stamps were made of Sylgard 184™ (Dow Corning), cured against the bottom of polystyrene dishes (Falcon).

Si structures for μFNs and subtractive printing were microfabricated. Microwells were made by photopatterning a resist, which had been spin-coated onto a Si(100) wafer, and by anisotropically etching Si with KOH using the resist as a mask. The resist was removed, and the top and bottom surfaces of the μwells were rendered hydrophobic by microcontact-printing perfluorodecyltrichlorosilane (Gelest). The μwells had an access area of 400×400 μm^2, a contact area of 100×100 μm^2 with the substrate, and a density of 100 wells on a 5×5 mm^2 area.

PDMS stamps were oxidized using an O_2 plasma for 10 s, placed in a 10% solution of APTS in water (acidified to pH 6 with acetic acid), and refluxed for 1 h at 80 °C. The stamps were then thoroughly rinsed with water, and covered with a 1 mM solution of BS^3 in water for 10 min. The stamps were rinsed with water once more, dried under a stream of N_2, and used immediately.

The μwells (Figure 2) were placed on an activated stamp, and filled with ~50 nL of a $200\,\mu g\,mL^{-1}$ solution of capture proteins using a micropipetting robot (Probot, BAI GmbH). After 10 min, the μwells were carefully rinsed with water and separated from the stamp under a 1% solution of BSA in PBS. Unreacted BS^3 was quenched by leaving the solution of BSA for 10 min. Irrespective of the preparation method of the α-stamps, loosely bound capture proteins were removed in one print onto a polystyrene surface.

Lines of proteins were deposited onto an activated stamp using μFNs (Figure 3) and 200 $\mu g\ mL^{-1}$ solutions of proteins in PBS. The μFN was removed from the PDMS stamp under a 1% solution of BSA in PBS. The patterned PDMS stamp was then contacted with a flat activated stamp for 10 min. Both stamps were separated, and the activated stamp, which retained the proteins, was exposed to a 1% BSA solution for 10 min.

Flat PDMS stamps were exposed to a 50 $\mu g\ mL^{-1}$ solution of capture antigens for 15 min to form a monolayer of capture proteins (Figure 4). After rinsing the stamps with PBS and water, and drying them under a N_2 flow, parts of the monolayer were removed by contacting the stamp with a structured Si substrate (3×3 μm^2 posts of 100 nm height) for a few seconds. The patterned layer of capture proteins was then transferred to an activated stamp during a 10-min-long contact. These steps were repeated to add arrays of different types of capture proteins onto the α-stamp. Affinity stamps were finally

exposed to a 1% solution of BSA in PBS for 10 min. They were stored in PBS, and inked for 30 min with a 50 μg mL^{-1} solution of target molecules (anti-chicken antibodies and anti-goat antibodies) in PBS with 1% BSA. After washing with PBS and water, and drying under a stream of N_2, the inked α-stamps were printed on a microscope slide for 10 s.

Fluorescence microscope images were acquired using a Labophot-2 microscope (Nikon) equipped with an ST-8 CCD camera (SBIG, Santa Barbara, CA) cooled to 0 °C. Fluorescence data from TRITC and FITC were acquired separately, and recombined with arbitrary colors. AFM images were obtained using a Nanoscope III AFM (Digital Instruments, Santa Barbara, CA) operating in taping mode with ultrasharp tips (Nanosensors, Germany).

Microarrays of antigoat antibodies printed on glass were exposed for 30 min to a PBS solution containing 1% of BSA and 2 μg mL^{-1} of goat antibody labelled with 10-nm-sized gold clusters. The glass slide was washed with PBS and water, and dried. A HQ silver reactant from Nanoprobes (Yaphank, NY) was used for the staining of the gold.